Scientia Horticulturae 231 (2018) 31–35

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Chlorophyll fluorescence and oxidative stress endpoints to discriminate olive cultivars tolerance to drought and heat episodes

Maria Celeste Dias[a,b,*], Sandra Correia[a], João Serôdio[c], Artur Manuel Soares Silva[b], Helena Freitas[a], Conceição Santos[d]

[a] Centre for Functional Ecology (CFE), Department of Life Science, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[b] QOPNA and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Department Biology & CESAM, University of Aveiro, Campus Universitário de Santiago 3810-193, Aveiro, Portugal
[d] Department of Biology, Faculty of Sciences, LAQV/REQUIMTE, University of Porto, Rua do Campo Alegre 4169-007, Porto, Portugal

ARTICLE INFO

Keywords:
Olea europaea
Photosynthesis
Total antioxidant capacity

ABSTRACT

Climate change is increasing the frequency of heat waves accompanied by drought episodes. These challenges are increasing in the Mediterranean basin, where *Olea europaea* L. has an important ecological and economic role. Olive breeding programs have been focused on highly productive cultivars, while ancient cultivars may present higher tolerance to drought and heat resilience. Therefore, it is important to select traditional cultivars that may give reliable performances under the emerging climate change scenarios. In the present work, the differential physiological response of economically important traditional Portuguese olive cultivars, Cobrançosa, Cordovil de Castelo Branco (C.C. Branco), and Cordovil de Serpa (C. Serpa), to drought combined with heat are evaluated. Stress treatment had lowest impacts on water status in Cobrançosa. Also, this cultivar was less affected regarding pigments content, maximum and effective quantum yield of photosystem II (F_v/F_m and Φ_{PSII}) and exhibited higher ability to trigger an antioxidant response. C.C. Branco was the most sensitive cultivar in response to pigments (carotenoids), F_v/F_m and Φ_{PSII}, and cell membrane stability. Principal component analysis suggested that Cobrançosa has high potential to withstand climate change events, particularly drought combined with heat episodes, followed by C. Serpa and C.C. Branco.

1. Introduction

The emerging climate change scenarios, such as increasing frequencies of heat waves accompanied by variations in the precipitation patterns, exert a dramatic challenge for agriculture, particularly for most crop-trees of the Mediterranean region (IPCC, 2014). Olive (*Olea europaea* L.) is one of the most important crop in the Mediterranean basin, with high economic and ecological value. This species presents some physiological and morphological plasticity to respond to the climate variability of the Mediterranean region, such as an efficient osmotic adjustment, leaf anatomical modifications, a good regulation of stomata aperture, high water uptake capacity and hydraulic lift and reverse flow (Fernández, 2014). Despite these adaptive capacities, olive response to the emerging combination of several stresses remains unknown and some reports highlighted its putative negative effects on productivity, and olive and oil quality (Martinelli et al., 2013).

Drought or heat can reduce photosynthesis, promote stomatal closure, induce photosynthetic pigments loss and oxidative stress. Ultimately olive plants may undergo a decreased growth and productivity (Koubouris et al., 2015). Being specific organelles for electron transfer, chloroplasts are a major source of reactive oxygen species (ROS), and particularly susceptible to oxidative stress. Under stress conditions, light energy absorption greatly exceeds the one required for photochemistry which may increase the generation/accumulation of ROS, causing oxidative damages. However, plants have evolved several mechanisms to prevent ROS formation or scavenge the unavoidably formed ROS (Pintó-Marijuan and Munné-Bosch, 2014). For instances, light absorption can be reduced or the extra photons' energy can be dissipated through photochemical and non-photochemical mechanisms. Also, plants possess an antioxidant system, composed by enzymatic and non-enzymatic antioxidants that can neutralise free radicals/ROS, reducing associated damages (Dias et al., 2014). Total antioxidant

Abbreviations: Chl, chlorophyll; CMP, cell membrane permeability; C, control plants; F_v/F_m, maximum quantum yield of photosystem II; F_o, minimum fluorescence; F_m, maximum fluorescence; F_s, steady-state fluorescence of light adapted samples; F_m', maximum fluorescence of light adapted samples; NPQ, non-photochemical quenching; PSII, photosystem II; qP, photochemical quenching; ROS, reactive oxygen species; RWC, relative water content; S, stress treatment; Φ_{PSII}, effective quantum yield of photosystem II

* Corresponding author at: Centre for Functional Ecology (CFE), Department of Life Science, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.
E-mail address: celeste.dias@uc.pt (M.C. Dias).

https://doi.org/10.1016/j.scienta.2017.12.007
Received 28 September 2017; Received in revised form 13 November 2017; Accepted 5 December 2017
Available online 11 December 2017
0304-4238/ © 2017 Elsevier B.V. All rights reserved.

capacity reflects the combination of these diverse antioxidant strategies. The degree of drought tolerance has been correlated with the plant́s antioxidant capacity (Petridis et al., 2012).

Some Portuguese ancient cultivars are originated from arid regions and their commercial value is emerging to produce olive oil of "Protected Designation of Origin – PDO" as the case of Cobrançosa and Cordovil (Amaral et al., 2010). Some studies compared cultivars' ability for adaptation and production under drought conditions (e.g. Bacelar et al., 2004; Faraloni et al., 2011). Bacelar et al. (2004) and Pierantozzi et al. (2013) reported the susceptibility to water deficit of cultivars used in intensive production, such as Arbequina and Manzanilla, showing decreased photosynthesis and increased lipid peroxidation combined with less leaf anatomical features to prevent from water loss.

Impacts of heat stress have been less addressed (Aguilera et al., 2014; Koubouris et al., 2015), while responses to combined stresses (e.g. drought + heat) remain to unveil. Considering that some ancient cultivars, besides having a PDO importance, are identified by farmers as highly tolerant to drought and/or heat, it is important to study these cultivars for selection under an emerging climate change scenario. This work aims at characterizing and discriminating ancient olive cultivars tolerance to combined drought + heat shock, regarding impacts at the photosynthesis and oxidative stress level.

2. Material and methods

2.1. Plant material and stress exposure

Three representative Portuguese cultivars were chosen, the Cobrançosa, typically from the northeast, Cordovil de Serpa (C. Serpa) from the southern province of Alentejo and Cordovil de Castelo Branco (C.C. Branco) from the center of Portugal. Two-year-old potted (5L) plants were watered to 100% field capacity and plants with similar height were randomly divided in two groups: Group 1–control conditions (C, $n = 6$ plants), plants well-watered; and Group 2 – water deficit and heat shock condition (S, $n = 6$ plants), water was withholding for 20 days and in the last day plants were exposed to 45 ± 2 °C for 4 h. The experiment was conducted in a climatic chamber with a relative humidity of 40% and a temperature of 45 ± 2 °C. Light in the climatic chamber was provided by Osram cool white fluorescent lamps that gave an intensity of 800 μmol m^{-2} s^1. After stress exposure chlorophyll *a* fluorescence, water status and cell membrane permeability were evaluated. Additionally, leaf samples were collected, fast-frozen in liquid nitrogen and stored at −80 °C for further analysis of pigments and total antioxidant capacity.

2.2. Leaf water status and chlorophyll content determination

Plant water status was assessed through the measurement of the leaf relative water content, %RWC = 100 × (fresh weight − dry weight)/(turgid weight − dry weight). Pigments were quantified as described in Dias et al. (2014). Briefly, frozen leaves were homogenized with acetone:Tris 50 mM (80:20, v/v, pH of 7.8), centrifuged and the absorbance was read in a spectrophotometer Genesys 10-uvS (Thermo Fisher Scientific Inc., Waltham, USA) at 663, 537, 647 and 470 nm.

2.3. Chlorophyll a fluorescence parameters

Photosynthetic activity was assessed through Pulse Amplitude Modulation (PAM) fluorometry using a portable PAM fluorometer Portable Junior-PAM, Gademann Instruments GmbH, Germany as described by Serôdio et al. (2013). Leaves were adapted to dark during 30 min, and then a weak-intensity modulated light pulse (blue light emitted by a LED-lamp peaking at 470 nm, half-bandwidth of 31 nm) was applied to determinate the minimum fluorescence (F_o) followed by a saturating pulse (> 5000 μmol m^{-2} s^{-1} for 0.7 s) to determine the maximum fluorescence (F_m). Then, leaves were adapted to light for 5 min and steady-state fluorescence (F_s) and the maximal fluorescence level F_m' were determined. Actinic light was turned off and the minimal fluorescence level $F_{o'}$ was measured. Maximum and effective quantum yield of photosystem II (PSII) [$F_v/F_m = (F_m - F_o)/F_m$ and $\Phi_{PSII} = (F_m' - F_s)/F_m'$, respectively], photochemical quenching [$qP = (F_m' - F_t)/(F_m' - F_o')$] and non-photochemical quenching [$NPQ = (F_m - F_m')/(F_m')$] were calculated.

2.4. Cell membrane permeability and total antioxidant capacity

Electrolyte leakage was used to determine cell membrane permeability (CMP) according to Farooq and Azam (2006). Briefly, leaves were immersed in de-ionized water and incubated overnight on a rotary shaker. The electrical conductivity (L_t/L_0) of solutions were read before (L_t) and after autoclaving (L_0) the samples (121 °C, 10 min). For total antioxidant capacity (TAC), leaf frozen samples (~100 mg) were homogenized with methanol (1.25 ml), sonicated in a 40 °C bath over 30 min and centrifuged (15,000 × g, 15 min, 4 °C). Then, the supernatant was added to an ABTS (2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) solution and the absorbance recorded at 734 nm (Re et al., 1999). TAC was calculated using a gallic acid standard curve ($y = 0.00036x + 0.057$, $r^2 = 0.99$).

2.5. Statistical analysis

Data were analysed by One-Way Analysis of Variance followed by a multiple comparison test (Holm-Sidak Test). Principal component analysis (PCA) was performed with CANOCO for Windows v4.02.

3. Results

3.1. Effect of stress on relative water content, pigments, photosynthesis, cell membrane permeability and total antioxidant activity

No significant changes were found on the RWC between control and stress treatment for Cobrançosa (Fig. 1A). However, for the other cultivars, stress treatment induced a significant reduction of the RWC (C. Serpa: 22% and C.C. Branco: 20%) in comparison to the respective control.

Carotenoids decreased significantly only in C.C. Branco plants when exposed to stress (Fig. 1B). Under control conditions, C. Serpa showed the lowest ($P < 0.05$) level of carotenoids. Stress exposure induced a significant decline in the content of chlorophylls in all cultivars when compared to the respective control (Fig. 1C, D).

Maximum quantum yield of PSII (F_v/F_m) in Cobrançosa and C. Serpa were not affected ($P > 0.05$) by stress treatment, but in C.C. Branco, stress decreased significantly this parameter when compared to the respective control (Fig. 1E). However, for all cultivars, stress treatment reduced significantly Φ_{PSII} (Fig. 1F). When compared to the respective control, the Φ_{PSII} drop was higher in C.C. Branco (61%) than in Cobrançosa or C. Serpa (41% and 45%, respectively). Also, qP decreased significantly in all cultivars when exposed to stress (Fig. 1G). No significant differences were found in the NPQ between control and stress treatment (Fig. 1H).

Cell membrane permeability increased ($P < 0.05$) in all cultivars (C.C. Branco: 38%, Cobrançosa: 53% and C. Serpa: 62%) after stress exposure (Fig. 1 I). However, plants from the C.C. Branco cultivar showed the highest ($P < 0.05$) values of CMP. Also, stress increased ($P < 0.05$) the TAC in all cultivars: Cobrançosa and C.C. Serpa increased ~70%, while in C.C. Branco increased only 46%, comparatively to the respective control (Fig. 1J).

3.2. Principal component analysis (PCA)

Principal component analysis was carried out to identify parameters that can best describe olive cultivars physiological performance upon

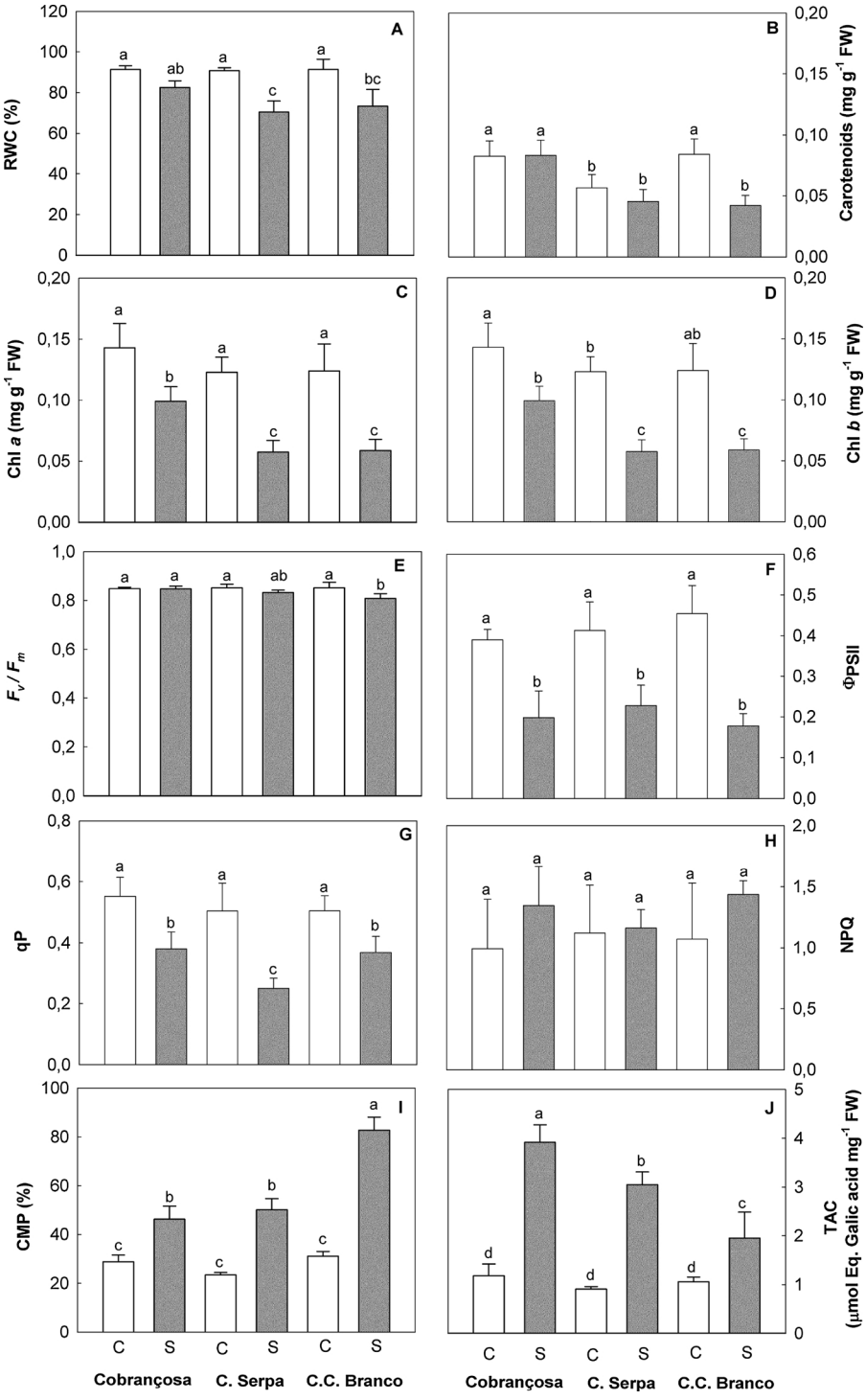

Fig. 1. A – Relative water content (RWC), B – carotenoids content, C – chlorophyll *a* content, D – chlorophyll *b* content, E – maximum quantum yield of photosystem II (F_v/F_m), F – effective quantum yield of photosystem II (Φ_{PSII}), G – photochemical quenching (qP), H – non photochemical quenching, I – cell membrane permeability (CMP), and J – total antioxidant capacity (TAC) in three *O. europaea* cultivars, under control (C) and drought combined with heat (S). Values are mean ± SD ($n = 6$). Different letters indicate statistical differences between treatments ($P < 0.05$).

stress exposure (Fig. 2). Control plants were all located on the left side, while stress plants were located on the right side, and forming three well defined groups that represents each cultivar. This separation between control and stress suggests a clear homogeneity in the physiological status of plants. The first component (X-axis) was determined mostly by the RWC, chlorophylls, and photosynthetic parameters (F_v/F_m, Φ_{PSII}, qP, NPQ) and CMP. The RWC, F_v/F_m, Φ_{PSII}, qP and pigments were positive correlated, and negative correlated with CMP, TAC and NPQ.

4. Discussion

In general, olive plants are well adapted to the harsh Mediterranean conditions showing several mechanisms to tolerate extremely low RWC and to avoid excessive water loss (Fernández, 2014). Leaf relative water content (RWC) is a commonly used indicator to evaluate plant water status and drought resistance (Faraloni et al., 2011). The combined stresses reduced the water status of all olive cultivars, but in C. Serpa and C.C. Branco values were below 80%, a threshold reported as indicator of a severe stress condition, while Cobrançosa plants maintained a RWC above this value, indicating only moderate stress (Bacelar et al., 2006; Dias et al., 2014). Our data support previous comparative

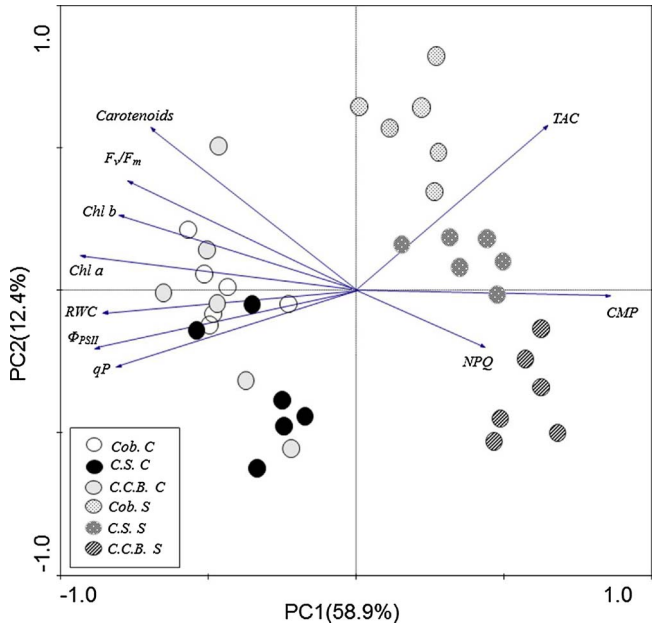

Fig. 2. PCA biplot of the physiological data in *O. europaea* cultivars in the experimental set-up. Loading plot for the first axis (explained 58.3% of the total variance) and second axis (explained 11.4% of the total variance). C – control; C.C.B. – Cordovil de Castelo Branco; Cob – Cobrançosa; C.S. – Cordovil de Serpa; and S – stress (drought and heat shock).

studies, where Cobrançosa showed higher tolerance to drought than Madural cultivar (Bacelar et al., 2004, 2006). In both cultivars RWC was reduced to ~85%, which was accompanied by increased levels of soluble sugars contributing to osmotic adjustment (Bacelar et al., 2006). Moreover, these authors demonstrated that Cobrançosa exhibit higher tissue elasticity, thicker cuticle and trichome layers promoting increased protection against water loss compared to Negrinha or Manzanilla cultivars (Bacelar et al., 2004).

Regarding photosynthetic and oxidative data, also Cobrançosa was less affected than the other cultivars, and simultaneously showed high ability to trigger an antioxidant response and low CMP. C.C. Branco was the most sensitive regarding decreases in pigments (carotenoids), F_v/F_m and Φ_{PSII}, also accompanied by higher CMP. Curiously, PCA scores of the control plants showed undistinguishable variations among the three cultivar populations, but in the stress conditions the separation of the cultivars was notorious, thus confirming a profile of tolerance Cobrançosa > C. Serpa > C.C. Branco (Fig. 2).

The differential decline of RWC in all cultivars in response to stress impaired Φ_{PSII}, a parameter that provides information of the rate of linear electron transport and so an indication of overall photosynthesis (Murchie and Lawson, 2013). Nevertheless, also qP and chlorophyll *a* reduction contributed to Φ_{PSII} decrease (Fig. 2). Despite the reduction in F_v/F_m for C.C. Branco the values were maintained within the range of ~0.8, typical of healthy plants (Dias et al., 2014).

Contrary to the reported in Coratina and Biancolilla olive cultivars exposed to drought (Sofo, 2011), in the present work, NPQ was not the main protection mechanism (Fig. 2) to prevent ROS production (Pintó-Marijuan and Munné-Bosch, 2014). This suggests that other mechanisms take place to protect olive plants to stress damages. A relationship between enhanced plant antioxidant activity and increased resistance to stresses was reported for several Mediterranean species, including *O. europaea* (Corcuera et al., 2012; Petridis et al., 2012). In the present work, TAC increased in response to stress and was positively correlated with CMP (Fig. 2). However, TAC enhancement in all cultivars was not enough to control putative oxidative damages, which may have contributed to increase membrane permeability (Demidchik et al., 2014) in all cultivars. Nevertheless, regarding membrane permeability, different levels of susceptibility were noticed between the three cultivars, being C. Serpa and C.C. Branco most sensitive (Fig. 2). In Cobrançosa, TAC contributes more to oxidative damage protection (Fig. 2). Our data are in agreement with Bacelar et al. (2006) showing that under drought conditions Cobrançosa possesses better protection against oxidative stress (low levels of lipid peroxidation and increased total thiol concentration) compared to Madural or Verdeal Transmontana cultivars.

As reported for several Mediterranean species, including olive (Bacelar et al., 2006; Boughalleb and Hajlaoui, 2011; Vasques et al., 2016), chlorophyll and, in a less extent, carotenoids were negatively affected by stress imposition in all cultivars, particularly in C. Serpa and C.C. Branco (Fig. 2). Carotenoids, besides their function as accessory light-harvesting pigments, also have an important role as antioxidants protecting chlorophylls from photooxidative destruction (Kataria et al., 2014). The combined stress affected more markedly the levels of these pigments in C.C. Branco, and the direct correlation between carotenoids and chlorophylls support that chlorophyll loss may be a consequence of carotenoids reduction. Chlorophyll loss, due to pigment (or its precursors) degradation, may be a consequence of oxidative stress increases (Pintó-Marijuan and Munné-Bosch, 2014), which is supported by the negative correlation found between chlorophylls and CMP (Fig. 2). Moreover, it may represent an adaptative feature to cope with stress, particularly in species usually exposed to excess of excitation energy as olive trees, resulting in lower leaf light absorbance that contribute to photoprotection (Vasques et al., 2016). The fact that C.C. Branco F_v/F_m and Φ_{PSII} were negatively affected, simultaneously with chlorophyll and carotenoids also suggests that these stresses induced damages in the photosynthetic apparatus of this cultivar, which supports its higher susceptibility.

In conclusion, even though olive has a high degree of tolerance to drought when compared with other species, our results demonstrate that drought combined with a heat shock affected the physiological performance of all cultivars, reducing water status and Φ_{PSII}. However, cultivars displayed differential response to stresses as could be seen by the range of effects on CMP and TAC, being Cobrançosa the cultivar least affected. Also the PCA demonstrated higher homogeneity of each cultivar population under stress, with high discrimination by TAC and CMP. Finally, our data suggest that Cobrançosa may cope better with climate change events, particularly drought and heat, and could be more suited to be planted in regions that are prone to these stresses.

Acknowledgements

This work was financed by FCT/MEC through national funds and the co-funding by the FEDER, within the PT2020 Partnership Agreement, and COMPETE 2010, within the projects UID/BIA/04004/2013, UID/QUI/00062/2013 and UID/QUI/50006/2013. FCT supported MCDias (SFRH/BPD/100865/2014) and SCorreia (SFRH/BPD/91461/2012). The authors declare that they have no conflict of interest.

References

Aguilera, F., Ruiz, L., Fornaciari, M., Romano, B., Galan, C., Oteros, J., Ben, D.A., Msallem, M., Orlandi, F., 2014. Heat accumulation period in the Mediterranean region: phenological response of the olive in different climate areas (Spain, Italy and Tunisia). Int. J. Biometeorol. 58, 867–876.

Amaral, J.S., Mafra, I., Oliveira, M.B.P.P., 2010. Characterization of three Portuguese varietal olive oils based on fatty acids, triactlglycerols, phytosterols and vitamin E profiles: application of chemometrics. In: Preedy, V.R., Ross, R. (Eds.), Olives and Olive Oil in Health and Disease Prevention. Watson Academic Press, USA.

Bacelar, E.A., Correia, C.M., Moutinho-Pereira, J., Gonçalves, B.C., Lopes, J.I., Torres-Pereira, J.M.G., 2004. Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. Tree Physiol. 24, 233–239.

Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Gonçalves, B.C., Ferreira, H.F., Correia, C.M., 2006. Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. Plant Sci. 170, 596–605.

Boughalleb, F., Hajlaoui, H., 2011. Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). Acta Physiol. Plant. 33, 53–65.

Corcuera, L., Gil-Pelegrin, E., Notivol, E., 2012. Aridity promotes differences in proline and phytohormone levels in Pinus pinaster populations from contrasting environments. Trees 26, 799–808.
Demidchik, V., Straltsova, D., Medvedev, S.S., Pozhvanov, G.A., Sokolik, A., Yurin, V., 2014. Stress-induced electrolyte leakage: the role of K+-permeable channels and involvement in programmed cell death and metabolic adjustment. J. Exp. Bot. 65, 1259–1270.
Dias, M.C., Oliveira, H., Costa, A., Santos, C., 2014. Improving elms performance under drought stress: the pretreatment with abscisic acid. Environ. Exp. Bot. 100, 64–73.
Faraloni, C., Cutino, I., Petruccelli, R., Leva, A.R., Lazzeri, Z., Torzillo, G., 2011. Chlorophyll fluorescence technique as a rapid tool for in vitro screening of olive cultivars (*Olea europaea* L.) tolerant to drought stress. Environ. Exp. Bot. 73, 49–56.
Farooq, S., Azam, F., 2006. The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties. J. Plant Physiol. 163, 629–637.
Fernández, J.-E., 2014. Understanding olive adaptation to abiotic stresses as a tool to increase crop performance. Environ. Exp. Bot. 103, 158–179.
IPCC, et al., 2014. Climate change 2014 impacts, adaptation, and vulnerability. part A: global and sectoral aspects. In: Field, C.B. (Ed.), Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
Kataria, S., Jajoo, A., Guruprasad, K.N., 2014. Impact of increasing ultraviolet-B (UV-B) radiation on photosynthetic processes. J. Photochem. Photobiol. B 137, 55–66.
Koubouris, G.C., Kavroulakis, N., Metzidakis, I.T., Vasilakakis, M.D., Sofo, A., 2015. Ultraviolet-B radiation or heat cause changes in photosynthesis: antioxidant enzyme activities and pollen performance in olive tree. Photosynthetica 53, 279–287.
Martinelli, F., Remorini, D., Saia, S., Massai, R., Tonutti, P., 2013. Metabolic profiling of ripe olive fruit in response to moderatewater stress. Sci. Hortic. 159, 52–58.
Murchie, E.H., Lawson, T., 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J. Exp. Bot. 64, 3983–3998.
Petridis, A., Therios, I., Samouris, J., Tananaki, C., 2012. Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. Environ. Exp. Bot. 79, 37–43.
Pierantozzi, P., Torres, M., Bodoira, R., Maestria, D., 2013. Water relations, biochemical—physiological and yield responses of olive trees (*Olea europaea* L. cvs. Arbequina and Manzanilla) under drought stress during the pre-flowering and flowering period. Agric. Water Manage. 125, 13–25.
Pintó-Marijuan, M., Munné-Bosch, S., 2014. Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: advantages and limitations. J. Exp. Bot. 65, 3845–3857.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.
Serôdio, J., Ezequiel, J., Frommlet, J., Laviale, M., Lavaud, J., 2013. A method for the rapid generation of nonsequential light-response curves of chlorophyll fluorescence. Plant Physiol. 163, 1087–1088.
Sofo, A., 2011. Drought stress tolerance and photoprotection in two varieties of olive tree. Acta Agric. Scand. B—Soil Plant Sci. 61, 711–720.
Vasques, A.R., Pinto, G., Dias, M.C., Correia, C.M., Moutinho-Pereira, J.M., Vallejo, V.R., Santos, C., Keizer, J.J., 2016. Physiological response to drought in seedlings of *Pistacia lentiscus* (mastic tree). New Forest 47, 119–130.